Docket No. 49632(71699)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Pamela L. Zeitlin

Serial No.: 09/523,776

Filed: March 11, 2000

For: MODULATION OF PROTEIN EXPRESSION USING CARBOCYCLIC ARYL ALKENOIC ACID DERIVATIVES

Examiner: Shengjun Wang

Art Unit: 1617

Mail Stop: Amendment

Commissioner for Patents

P.O. Box 1450

Alexandria, VA 22313-1450

Sir:

DECLARATION UNDER 37 C.F.R. 1.132

I, Pamela L. Zeitlin, M.D., a citizen of the United States of America residing at 1808 South Road, Baltimore, Maryland 21209, hereby declare as follows:

1. I am a co-inventor of the subject matter described and claimed in the patent application U.S.S.N. 09/523,776, filed on March 11, 2000 and otherwise identified above.

2. I have read and understood the Office Action dated October 3, 2006 and the references cited in the Office Action in the above case.

3. The following experiments were conducted by me or under my supervision, to

compare the effect of trans-styrylacetic acid (t-SAA, aka 4-phenyl-Δ3-transbutenoic acid) versus cinnamic acid and 4-phenyl butyric acid (4-PBA) in ΔF508-CFTR protein expression in model cells of cystic fibrosis..

5. The experiment was conducted as a pulse-chase protocol as described in our recent publication Vij, N, Fang S, and PL Zeitlin, Selective Inhibition of Endoplasmic Reticulum Associated-degradation Rescues ΔF508-Cystic Fibrosis Transmembrane Regulator and Suppresses Interleukin-8 levels: Therapeutic Implications. Journal Biological Chemistry. 281:17369-17378, 2006. The method is described on p. 17370 in the sections on Cell Culture, Transfection, and Metabolic Labeling and Immunoprecipitation and Immunoblotting. Native IB3-1 cells were treated with the indicated compounds: untreated control, vehicle DMSO control, 5 mM CA, SAA, and 4PBA for 48 hrs at 37 degrees C. The cells were rinsed three times, starved of methionine in methione-free and cysteine free Dulbecco's Modified Eagles Medium, pulsed 250 microcuries per ml ^{35}S-methionine/cysteine radiolabel (ICN Biomedical, Irvine, CA) for 30 min and chased with unlabeled 10 mM methionine and 4 mM cysteine in Dulbecco's Modified Eagles Medium for 2 hrs. The CFTR was immunoprecipitated as described. Cells were lysed with M-Per and 500 micrograms protein were incubated with 50 micrograms of Protein A/G agarose beads (Santa Cruz Biotechnology Inc) for 3 hrs at 4 degrees C. After this pre-clearing, 5 micrograms of rabbit anti CFTR 169 antibody was added. The protein/beads/antibody was incubated overnight at 4 degrees C. The mixtures were washed, eluted from beads as described and then separated on a 4-10% SDS gel. Gels were transferred, dried for 2 hrs and processed for autoradiographic imaging. The CFTR forms band B and band C were quantified by densitometry.

6. Exhibit A (attached) is an autoradiograph showing ΔF508-CFTR protein expression in model cells of cystic fibrosis treated with the indicated compounds as described above. ΔF508-CFTR is present in two bands, B and C. Band B corresponds to the immature core-glycosylated isoform of CFTR; Band C ~~corresponds to the mature complex-glycosylated~~

CFTR isoform. ΔF508-CFTR Bands B and C were quantitated by densitometry, and the quantitation is shown in Exhibit B (attached). Cells treated with trans-SAA produced increased amounts of both the immature and the mature forms of ΔF508-CFTR, consistent with an increase in CFTR production.

7. These results indicate that trans-SAA is surprisingly effective in promoting the trafficking of functional ΔF508-CFTR to the cell surface relative to cinnamic acid and 4-PBA. Based on this side by side comparison of trans-SAA to cinnamic acid and 4-PBA, it is our opinion that trans-SAA has unexpectedly superior activity relative to cited art compounds cinnamic acid and 4-PBA.

8 I, the undersigned Pamela L. Zeitlin, M.D., further declare that all statements made herein of my own knowledge are true and that all statements made upon information and belief are believed to be true, and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 101 of Title 18 of the United States Code and that such willful false statement may jeopardize the validity of the above identified application or any patent issuing thereon.

By: Pamela L. Zeitlin MD

Pamela L. Zeitlin, M.D.

Date: March 5, 2007

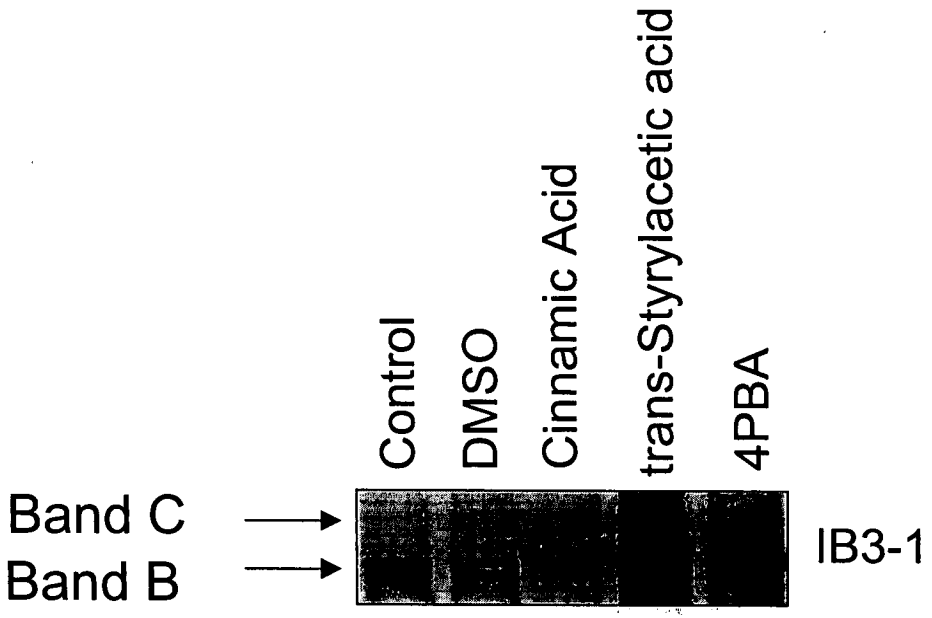

Exhibit A

Pulse: 30min
Chase: 2hrs

IB3-1

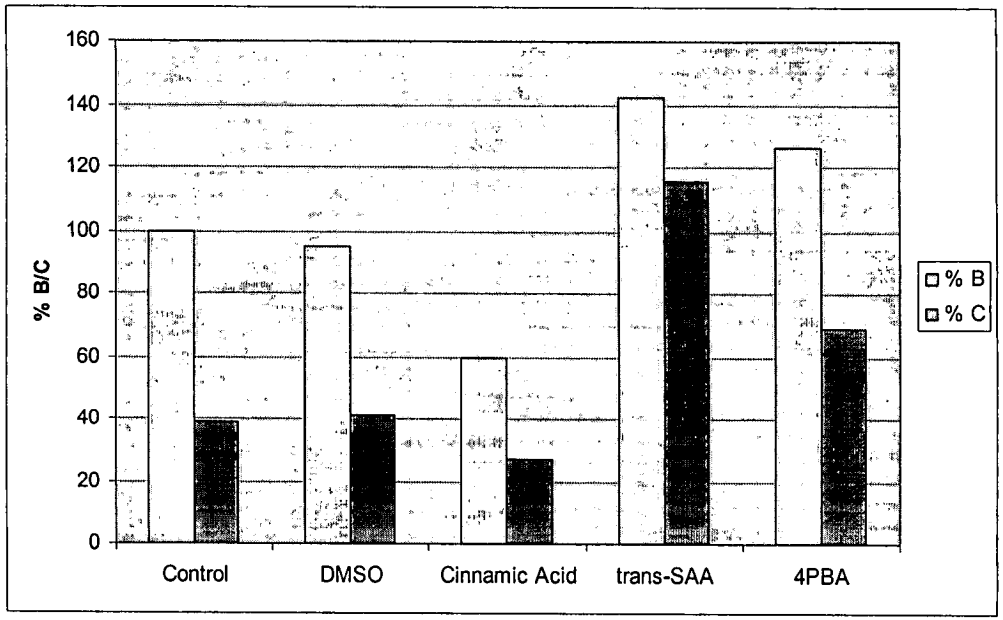

Exhibit B